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CLEC8A AND P-SELECTIN: NEW TUMOR MARKERS FOR DIAGNOSIS OF BREAST CANCER AND EVALUATION OF CHEMOTHERAPY EFFICIENCY IN SUPPRESSING IT

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Abstract

Background: Breast cancer is a global health problem with significant impacts on women's health worldwide(99). Understanding the mortality, prevalence, and incidence of breast cancer across geographic and demographic dimensions is an important epidemiological feature of breast cancer, as well as identifying risk factors that facilitate the development of appropriate public health policies(100). Breast cancer became the most diagnosed cancer among females worldwide in 2020, with approximately 2.26 million new cases reported. The global burden of breast cancer is projected to increase to more than 3,000,000 new cases annually by 2040. Globally, across ethnicities and regions, there are significant variations. Subjects and Methods: Over a period of seven months (from the beginning of October 2023 to the end of April 2024), 45 women were enrolled in the current study. The participants in the current study were divided into three groups based on their health status (women with breast tumors and healthy women), and depending on the type of tumor, the patients were divided into: women with malignant breast tumors and women with benign breast tumors. The first patients group consisted of 15 females with malignant breast tumors who participated in the work before starting chemotherapy and were followed up during receiving 3 consecutive doses of chemotherapy. The second patients group consisted of 15 females with benign tumors (enrolled as a pathological control group), while the healthy control group consisted of 15 females, their age ranged from 22 to 45 years. Sandwich enzyme linked immune sorbent assay (Sandwich ELISA) method was applied to evaluate C-Type Lectin Domain Family 8, member A (CLEC8A)and P-Selectin concentrations in the sera samples of the study individuals. Results: The result of the present work shows that there are no statistically significant differences in BMI in patients with different breast tumors when comparing the two groups together ($p=0.090$), as well as when comparing the group of patients with malignant tumors with the healthy control group ($p=0.081$), or when comparing the group of females with benign breast tumors with the healthy control group, the difference ($p=0.957$). The results showed a significant elevation in CLEC8A levels in the malignant tumor group comparison to the benign tumor group ($p=0.000$) as well as the health control group ($p=0.000$).Similarly, but with less statistical acceptability, a difference in CLEC8A concentration was observed when comparing the two control groups (patients with benign breast tumors and healthy females) together. When CLEC8A levels were evaluated during chemotherapy, a significant increase in the levels of this parameter was observed after receiving the first dose of chemotherapy, followed by a gradual decrease with the progression of doses. CLEC8A level was quickly decreased to a level lower than it was before starting treatment after receiving the second dose of chemotherapy. A significant decrease in the level of CLEC8A was observed after taking the last dose within the recommended course of chemotherapy compared to the levels of this lectin in the stage prior to starting the chemotherapy program. Statistical analysis using ANOVA test showed a significant increase ($p=0.000$) in P-Selectin levels in females with breast cancer when compared with benign breast tumor patients as well as healthy controls. When monitoring P-Selectin levels during the chemotherapy phase, a gradual decrease in the levels of this parameter was observed, proportional to the progress of the doses received by the patients, with a slight increase in the P-Selectin level recorded after the first dose of chemotherapy in only one patient, followed by a decrease in those values with the progress of the

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treatment phases as in the rest of the samples. The results showed a significant (p=0.000) difference when comparing the P-Selectin level after receiving the last dose with its level before starting chemotherapy, as the levels of this parameter decreased to their levels in the healthy control group. Significant positive correlations were observed when studying the relationship between CLEC8A and P-Selectin in the group of patients with breast cancer ($r = 0.712$ at $p<0.005$) and the group of healthy females ($r = 0.646$ at $p<0.005$). The individual efficiency (sensitivity) of the criteria evaluated in the current study for distinguishing between cancerous and benign breast tumors reached the highest level (100%), while the study established the highest specificity (100%) for both CLEC8A and P-Selectin. Combined CLEC8A and P-Selectin.in this study showed a similar maximum sensitivity for each parameters (100%). Conclusion: Accordingly, the results of the present work indicate the possibility of using one of the two criteria, CLEC8A or P-Selectin, alone to infer a benign breast tumor, as the levels of these two criteria remain within their limits in the sera of healthy people, and thus it can be asserted that the detected tumor is a non-cancerous tumor.

Key Words CLEC8A, P-Selectin, Breast Cancers, Chemotherapy

Introduction

Cancer is a group of disorders in which the body's cells grow, multiply and change without control, the lack of control over the ability of these cells to reproduce and they continue to divide and multiply abnormally instead of multiplying regularly or normally[(1)].A mass of excess tissue known as a tumor appears. These tumors may be benign (non-cancerous) or malignant (cancerous). Malignant tumors invade and destroy healthy tissue, and these malignant cells may spread to other places in front of the tumor site or to other organs[(2)]. Breast cancer is one of the leading causes of death in the world and is the most common coming women among all cancers, as result of several internal and external factors, breast cancer may occur and develop[(3)]. It is an advanced, multi-stage malignant tumor that affects women, and a small percentage of men breast cancer can occur because of changes in breast cells[(4)].Usually, breast cancer begins in the cells that make up the ducts that carry milk from the glands to the nipple, which are tubes. Cancer that begins in this area is called ductal carcinoma, a subtype of breast cancer. When a cancer occurs in the milk-producing glands, it is called lobular carcinoma[(5)]. There are also fewer common types that occur in breast cancer, such as Paget's disease, triple-negative breast cancer, inflammatory breast cancer, soft tissue sarcoma, and lymphoma[(6)]. It has been proven that 20 to 30% of breast cancer is due to modifiable factors, while 5 to 10% is due to genetic mutations and family history[(7)]. Breast cancer can develop in women who suffer from risk factors that increase the likelihood of infection, and women are more likely than men to develop breast cancer when breast cells are exposed to estrogen and progesterone[(8)],these factors help breast cancer grow. Breast cancer is a complicated disease by different biological and histological characteristics such as metastasis and invasiveness proto-oncogene activation also plays a role in tumor progression and formation[(9-11)].

C-Type Lectin Domain Family 8, Member(CELC8A) is a type II transmembrane homologous protein consisting of four distinct domains. The dimmers are linked between the molecules by disulfide bonds. The amino group is intracellular, located in a short cytoplasmic region, followed by an extracellular helical region, a transmembrane region, and a neck domain that binds to a conserved lectin-like domain (CTLD)[(12)]. The primary structure of CELC8A shows high genetic similarity across varied species and is therefore an inducible regulatory gene. Low-density lipoprotein-like oxidized lectin CELC8A, also known as LOX-1, SCAREI, OLRI[(13)].CELC8A receptor involved in inflammatory processes as well as cell activation and has a role in host response or infection through its interaction with pathogens[(14)]. CELC8A plays an interactive role with pathogens, participating in the inhibition of

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inflammatory processes and immune cells, suggesting a role in host response and infection as well as its involvement in immune inflammation and bacteria, fungi, and viruses in the immune response, in addition to facilitating protective immunity against tumors as well as a distinct role in the development of atherosclerosis[15].

Selectins are vascular adhesion molecules expressed on the surface of leukocytes, platelets, and endothelial cells, which infiltrate immune cells into surrounding tissues via the bloodstream during the first step. Selectins typically consist of a single EGF domain and a C-terminal lectin[16]. Selectins are divided into three types: E-, L-, and P-selectin. E-selectin is typically expressed on endothelial cells, While L-selectin is expressed primarily by leukocytes, But and P-selectin is expressed by platelets, as well as by inflamed endothelial cells, and translocated to the cell membrane within minutes [17].P-Selectin is also considered one of several proteins that bind to the venous mass and consists of about nine regulatory protein repeats, a human growth factor, an amino-terminal lectin domain, transmembrane sections, and small intracytoplasmic ends[18]. P-Selectin is produced on lamellipodium and endothelial cells and is encoded by SELP in humans[19]. P-Selectin is also considered the most important biomarker and that cycle in thrombotic events as well as the inflammatory cycle and its location on the endothelium. P-Selectin also has a noticeably short binding site and is not detectable in healthy conditions but is detected during venous mass degradation[20].It is stored in Weibel-Palade bodies and localizes to granules during exocytosis and is stimulated by thrombin when brought to the cell surface and remains for a brief time and is rapidly absorbed into the cell [21].

Materials and methods

The cohort study was applied in the present work with the aim of finding new tumor diagnostic markers for breast cancers and distinguishing them from non-cancerous breast tumors (benign breast tumors), first by evaluating the levels of lectins that have not been studied previously in breast tumors, as well as trying to obtain biochemical tools to evaluate the efficiency of chemotherapy in suppressing breast cancers.

The Study Population

Over a period of seven months (from the beginning of October 2023 to the end of April 2024), 45 women were enrolled in the current study. The participants in the current study were divided into three groups based on their health status (women with breast tumors and healthy women), and depending on the type of tumor, the patients were divided into: women with malignant breast tumors and women with benign breast tumors.

The first patients group consisted of 15 females with malignant breast tumors who participated in the work before starting chemotherapy and were followed up during receiving 3 consecutive doses of chemotherapy. Their ages ranged between 32 and 61 years, their weight ranged between 47 and 111 Kg, while their length ranged between 137 and 160 cm.

The second patients group consisted of 15 females with benign tumors (enrolled as a pathological control group), whose ages ranged from 15 to 52 years, their weight was ranged between 36 and 100 Kg, while their length ranged between 150 and 170 cm. while the healthy control group consisted of 15 females, their age ranged from 22 to 45 years.

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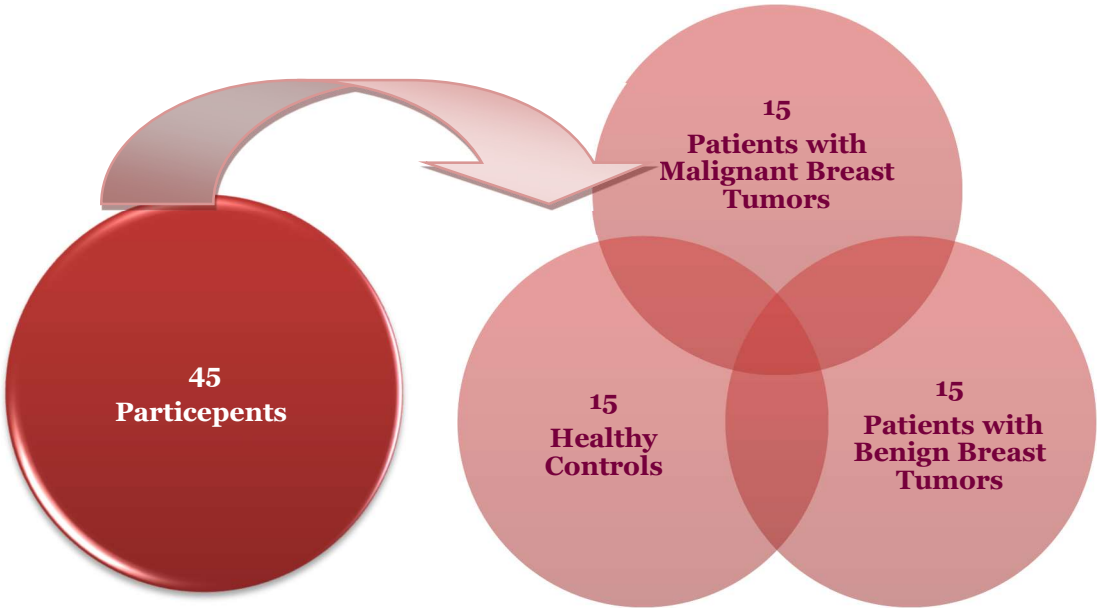

Figure 1: Distribution of the Study Individuals

Seven of patients with malignant tumor had a tumor location in the right breast and eight of them had left breast tumor location. The stages of the patients were divided between the second and the fourth, where they were divided as follows: 9 females were in the second (II) stage of cancer, 5 of them were in the stage third (III) and finally only one case of them was in the fourth stage (IV). All patients with malignant tumors except two were married and had 2-7 children.

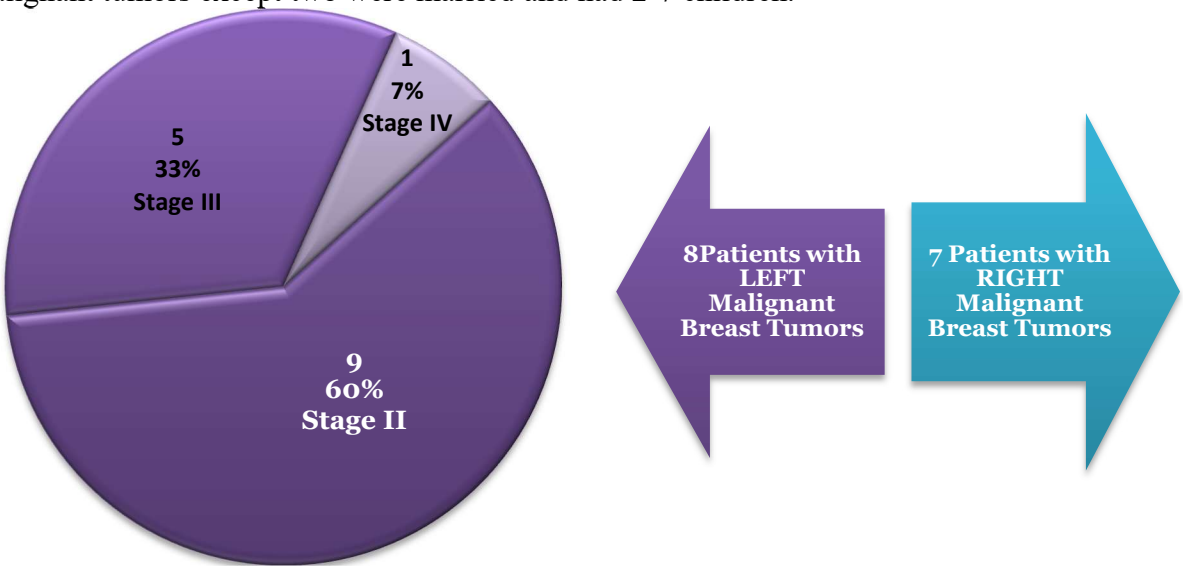

Figure 2: Distribution of Malignant Breast Tumors According to the Stage of Disease and Location of Tumor

Eight of the 15 patients with benign breast tumors had their tumors in the right breast and the remaining number had their tumors in the left breast. With the exception of only five cases, the patients with benign breast tumors were married and had 1-8 healthy births. In addition, all patients with benign breast tumors were not take contraceptives during the appearance of the tumor. Finally, some patients

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in this group had undergone cesarean section only as a surgical intervention before the occurrence.According to the questionnaire prepared based on the opinion of specialist doctors, which included complete information about age, place of residence, profession, period of onset of symptoms and medical history, the cases were collected.The samples of patients with malignant breast tumors were collected from the National Oncology and Hematology Hospital before receiving chemotherapy and were followed up during the chemotherapy period. The patients with cancerous breast tumors had undergone surgical treatment 3-5 weeks before receiving the first dose of chemotherapy.Samples of patients with benign breast tumors were collected from the Breast Cancer Early Detection Unit in Al-Sadr Medical City, while samples of the healthy control group were collected from the study community environment, such as postgraduate students and their relatives, as well as workers in the centers and hospitals from which the infected samples were collected.The current study required exclusion the following female cases:All participants (patients with breast tumors or healthy controls) who had suffered chronic diseases, *i.e.;* liver, renal, cardiovascular diseases, diabetes, hypertension and morbid obesity.Breast tumor patients who did have any types of tumors before diagnosis breast tumor.Patients whose disease symptoms coincided with taking oral or intravenous contraceptives or who took oral contraceptives for 3 consecutive years before the onset of symptoms.Cases who underwent surgery within 5 years of onset of symptoms, pregnant and smokers.

Assessment of C-Type Lectin Domain Family 8, Member and P-Selectin

Sandwich enzyme linked immune sorbent assay (Sandwich ELISA) method was applied to evaluate C-Type Lectin Domain Family 8, member A (CLEC8A)and P-Selectin concentrations in the sera samples of the study individuals.

The Statistical Analysis of the Data

The outcomes of the present study were analyzed through the statistical package for the social sciences (SPSS) version 26 software application statistical analysis system and excel (statistical package). The variables were illustrated by mean ± S.D, minimum, maximum, frequencies, and percentages. Graphics are presented using pie and bar charts. Inferential data analysis includes analysis of variance (ANOVA) test was applied to assess differences between the levels of the studied parameters. Pearson's correlation was applied to determine the relation among the parameters of the present study. The probability of deflection than controls are considered statistically significant if *p*-value is below 0.05. Receiver operating characteristic (ROC) curve was applied to present the sensitivity of the evaluated parameters. Combined sensitivity and specificity percentages were calculated according to biomedical statistical.

Results and Discussion

Topic of Age

According to the results of the analysis of variance (ANOVA) test, there were no statistical differences in age when comparing the two groups with breast tumors (p=0.054), and in the same manner when comparing the group with malignant breast tumors and healthy individuals (p=0.270). However, the results were contrary to what was previously mentioned when comparing the ages in the group of individuals with benign breast tumors with their counterparts in the group of healthy individuals, as the study showed the presence of significant differences (p=0.003) in age when comparing these two groups (**Table 1**).

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Table 1: Age of the Study Subgroups

Study Groups (n)	Age (Year) Mean ± S.D.	Min.-Max. Range	*p-value*
Malignant Tumors 15	44.333±8.673	32.00-61.00 29.00	**0.054 for MT *vs* BT**
Benign Tumors 15	35.466±17.747	15.00-82.00 67.00	**0.003 for MT *vs* C**
Controls 15	30.466±7.698	22.00-45.00 23.00	**0.270for BT *vs* C**

The mean difference is significant at the 0.05 level. MT: Malignant Tumors, BT: Benign Tumors, and C: Controls

Based on information from the information form used, the study did not find the effect of age on the incidence of cancer, where the injury included women in the third decade until the seventh decade, moreover; the study found that the genetic factor contributes significantly to the incidence of cancer at an early age where most of the patients had a family history of cancer.

With increasing age, the risk of breast cancer increases[22], and about 80% of new breast cancer cases are in women over the age of 50[23].Previous studies have shown molecular changes in age-specific patterns. Young breast cancer patients have a worse prognosis than older patients, and higher frequencies of aggressive clinical pathological feature[24].

Topic of Body Mass Index

The present work aims to study the effect of body massindex (BMI) on the occurrence of carcinogenesis. The result of the present work shows that there are no statistically significant differences in BMI in patients with different breast tumors when comparing the two groups together (p=0.090), as well as when comparing the group of patients with malignant tumors with the healthy control group (p=0.081), or when comparing the group of females with benign breast tumors with the healthy control group, the difference (p=0.957), as illustrated in **Table 2**.

Table 2: Body Mass Index (Kg/m^2) of the Study Groups

Study Groups (n)	BMI (Kg /m2) Mean ± S.D.	Min.-Max. Range	*p-value*
Malignant Tumors 15	31.572±7.013	24.320-47.420 23.090	**0.090 for MT *vs* BT**
Benign Tumors 15	25.921±8.916	24.160-43.630 19.470	**0.081 for MT *vs* C**
Controls 15	25.744±4.728	17.800-38.100 20.300	**0.957 for BT *vs* C**

The mean difference is significant at the 0.05 level. MT: Malignant Tumors, BT: Benign Tumors, and C: Controls

In general, as it was observed that there was no significant increase in the body mass index in the three groups, this indicates that there is no relationship between the BMI of patients with malignant breast tumors and the carcinogenicity of the cases recorded in the current study.The lowest BMI (17.800 Kg/m^2) was recorded for case in the benign tumor group, on the other hand; the highest value of BMI

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(47.420 Kg/m^2) was for a patient in cancerous breast tumor group.Obesity is widely recognized as a poor prognostic factor for breast cancer, despite being shown as a risk factor[25]. The link between breast cancer risk and body mass index (BMI) has gotten increasing attention in recent years, although the results are still debatable[26], overweight, and obesity have risks and a strong relationship with breast cancer[27].

Estimation of C-Type Lectin Domain Family 8 Member ALevels in the Study Groups

C-type lectin domain family 8member A (CLEC8A) levels were appraised in the sera samples of the malignant breast tumor, benign breast tumor and healthy individuals. The results showed a significant elevation in CLEC8A levels in the malignant tumor group comparison to the benign tumor group (p=0.000) as well as the health control group (p=0.000).Similarly, but with less statistical acceptability, a difference in CLEC8A concentration was observed when comparing the two control groups (patients with benign breast tumors and healthy females) together, as illustrated in **Table 3.**

Table 3: Levels of C-Type Lectin Domain Family 8 Member A(pg/mL) in the Sera Samples of theStudy Groups

Study Groups (n)	CLEC8A (pg/mL) Mean ± S.D.	Min.-Max. Range	*p-value*
Malignant Tumors 15	237.727±14.566	214.530-259.700 45.17	**0.000 for MT *vs* BT**
Benign Tumors 15	149.954±18.610	116.000-191.610 75.61	**0.000 for MT *vs* C**
Controls 15	136.654±18.960	92.120-167.000 74.88	**0.043 for BT *vs* C**

The mean difference is significant at the 0.05 level. MT: Malignant Tumors, BT: Benign Tumors, and C: Controls

The current study indicated that the highest CLEC8A level (259.70 pg/mL) was in a non-obese woman (BMI=26.563) in the fifties (42 years) with breast cancer, while the lowest CLEC8A level (92.12 pg/mL) was observed in a sample of a non-obese woman (BMI=26.839) from the healthy control group in the early fifties (40 years).

When CLEC8A levels were evaluated during chemotherapy, a significant increase in the levels of this parameter was observed after receiving the first dose of chemotherapy, followed by a gradual decrease with the progression of doses (**Figure 3**). CLEC8A level was quickly decreased to a level lower than it was before starting treatment after receiving the second dose of chemotherapy. A significant decrease in the level of CLEC8A was observed after taking the last dose within the recommended course of chemotherapy compared to the levels of this lectin in the stage prior to starting the chemotherapy program, as shown in **Figure 4**.

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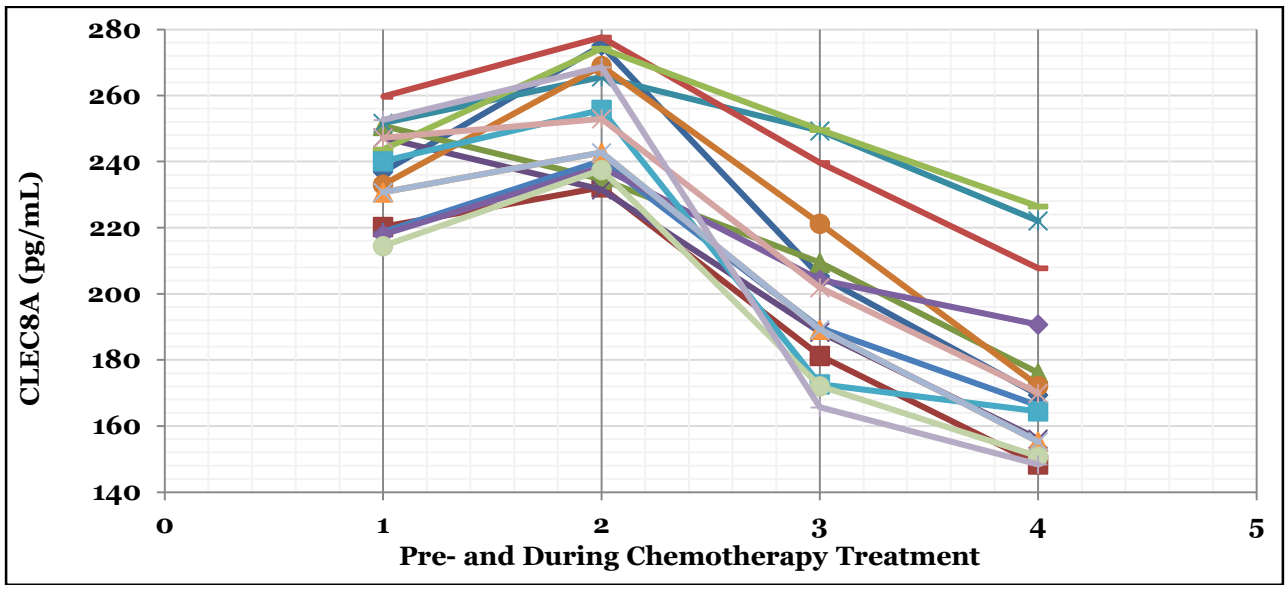

Figure 3: Follow-up of C-Type Lectin Domain Family 8 Member A Levels during Consecutive Chemotherapy

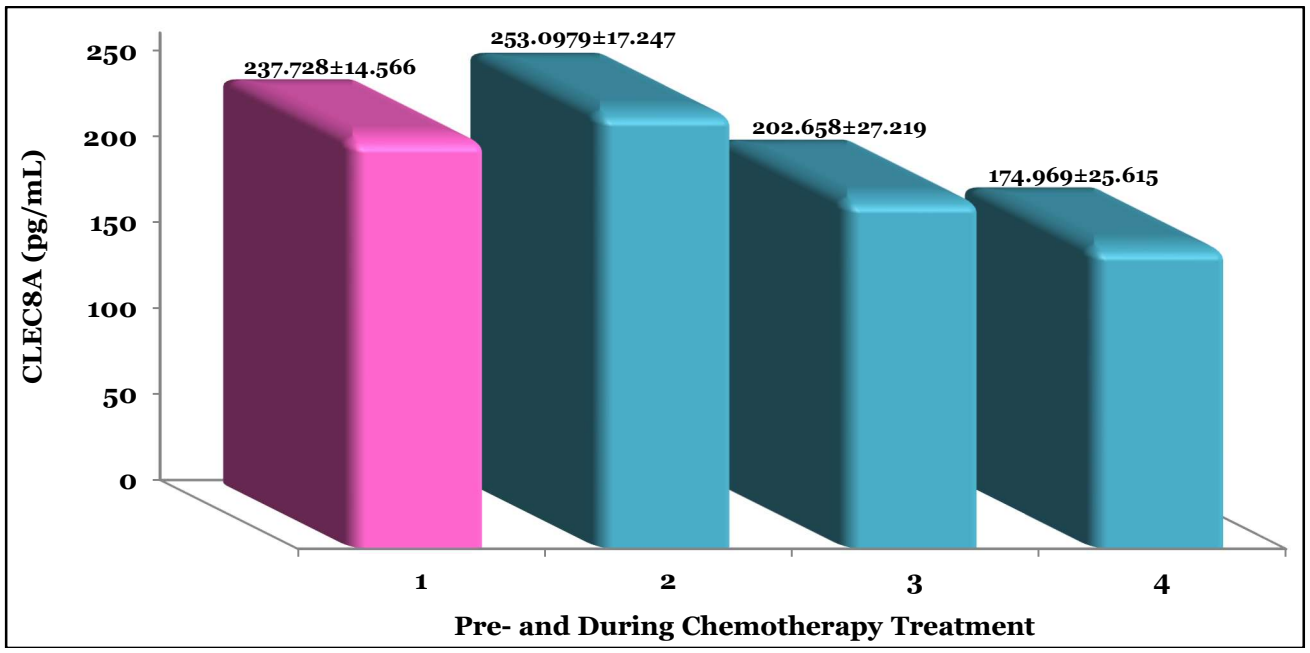

Figure 4: Comparison Levels of C-Type Lectin Domain Family 8 Member A in Cancerous Patients at Pre and Post Chemotherapy Treatment

The results of the current study showed an increase in CLEC8A levels in the serum of patients with malignant breast tumors when compared to individuals with benign breast tumors and healthy individuals. On the other hand, CLEC8A levels in the serum of patients with benign breast tumors remained higher than their levels in normal individuals. The results of the current work indicated a significant increase in lectin levels after receiving the first dose of chemotherapy, indicating a significant

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immune stimulation, followed by a decrease in its levels to the levels of that protein in the group of healthy individuals, indicating the success of chemotherapy in achieving the desired goal. CLEC8A is a C-type lectin receptor that is involved in inflammatory processes and immune cell activation, suggesting a role in infection or host response and the interactive cycle with pathogens[28]. Elevated serum CLEC8A levels are a poor prognostic factor in many cancers including breast cancer. CLEC8A levels are elevated in 57% of bladder and cervical cancer cells, 20% of colorectal cancer cells, 11% of breast cancer cells, and up to 10% of lung cancer cells. Furthermore, several studies have shown that elevated levels of CLEC8A expression are a major contributor to cancer cell progression. CLEC8A expression has been shown to be significantly elevated in various types of cancer where the role of CLEC8Alies in the proliferation, invasion, and migration of cancer cells as well as angiogenesis[29]. CLEC8A has no known enzymatic or catalytic activity in the cytoplasm and may require interaction with protein(s) for intracellular signaling. Following ligand uptake, CLEC8A stimulates pro-inflammatory signaling pathways that lead to ROS production, secretion of pro-inflammatory cytokines, and induction of apoptotic signals. Importantly, several studies have described the importance of CLEC8A in the development of various types of cancer. Functionally, CLEC8A induction by oxidized low density lipoproteins (ox-LDL), that recent research has shown their involvement in cancer, has been shown to stimulate TNF-α expression, tumor angiogenesis, tumor cell transvascular migration, and metastasis in breast cancers[30]. A literature search found no previous work for evaluating CLEC8A levels in breast cancer patients during chemotherapy.

Estimation of P-Selectin Levels in the Study Groups

Levels of P-Selectin concentration were evaluated in serum samples of the participants in the present study. Statistical analysis using ANOVA test showed a significant increase (p=0.000) in P-Selectin levels in females with breast cancer when compared with benign breast tumor patients as well as healthy controls. On the other hand; the results showed no statistical differences when comparing benign breast tumor patients and healthy controls, as shown in **Table 4**.

Table 4: Levels of P-Selectin (pg/mL) in the Sera Samples of the Study Groups

Study Groups (n)	P-Selectin (pg/mL) Mean ± S.D.	Min.-Max. Range	*p-value*
Malignant Tumors 15	190.424±8.413	169.910-198.860 28.950	**0.000 for MT *vs* BT**
Benign Tumors 15	135.634±12.676	112.030-157.660 45.630	**0.000 for MT *vs* C**
Controls 15	131.063±17.615	88.560-149.640 61.080	**0.869 for BT *vs* C**

The mean difference is significant at the 0.05 level. MT: Malignant Tumors, BT: Benign Tumors, and C: Controls

When monitoring P-Selectin levels during the chemotherapy phase, a gradual decrease in the levels of this parameter was observed, proportional to the progress of the doses received by the patients, with a slight increase in the P-Selectin level recorded after the first dose of chemotherapy in only one patient, followed by a decrease in those values with the progress of the treatment phases as in the rest of the samples (**Figure 5**).

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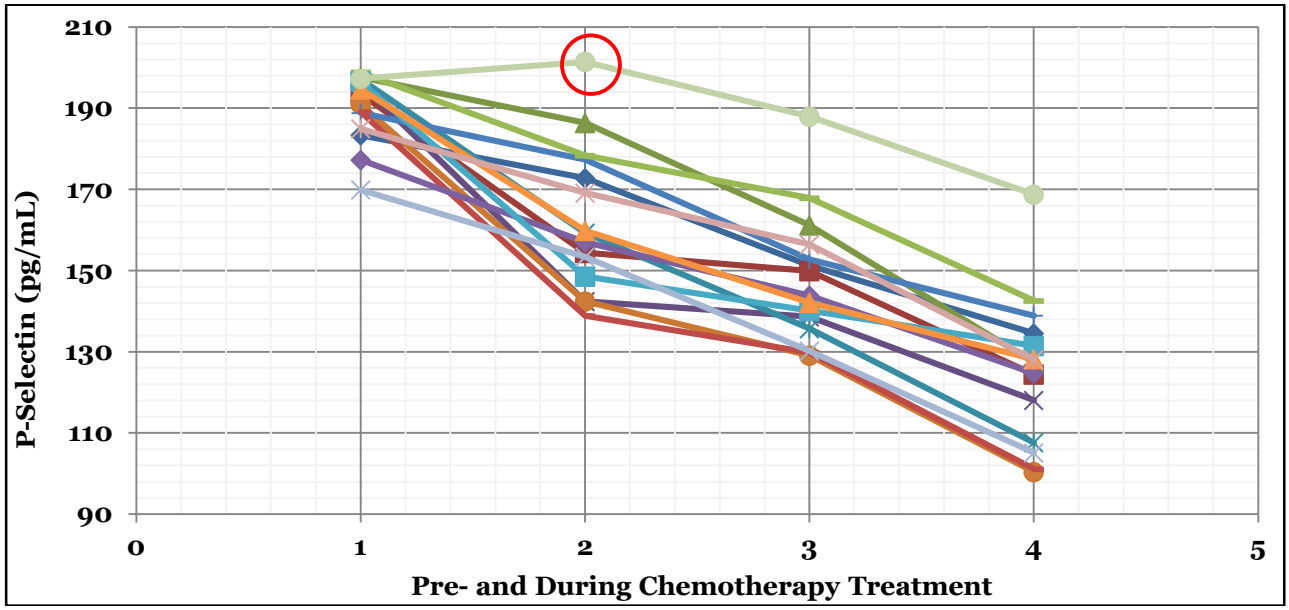

Figure 5: Follow-up of P-Selectin Levels during Consecutive Chemotherapy

Figure 6 shows a significant (p=0.000) difference when comparing the P-Selectin level after receiving the last dose with its level before starting chemotherapy, as the levels of this parameter decreased to their levels in the healthy control group. The gradual decrease in P-Selectin levels with the progression of chemotherapy may indicate the body's response to the treatment, moreover, it may be indicated by a decrease in the number of cancer cells, which leads to a decrease in the production of enzymes that stimulate the formation of P-Selectin. Based on the results obtained, P-Selectin can be used as an effective tool to monitor patients' response to treatment during successive stages of chemotherapy.

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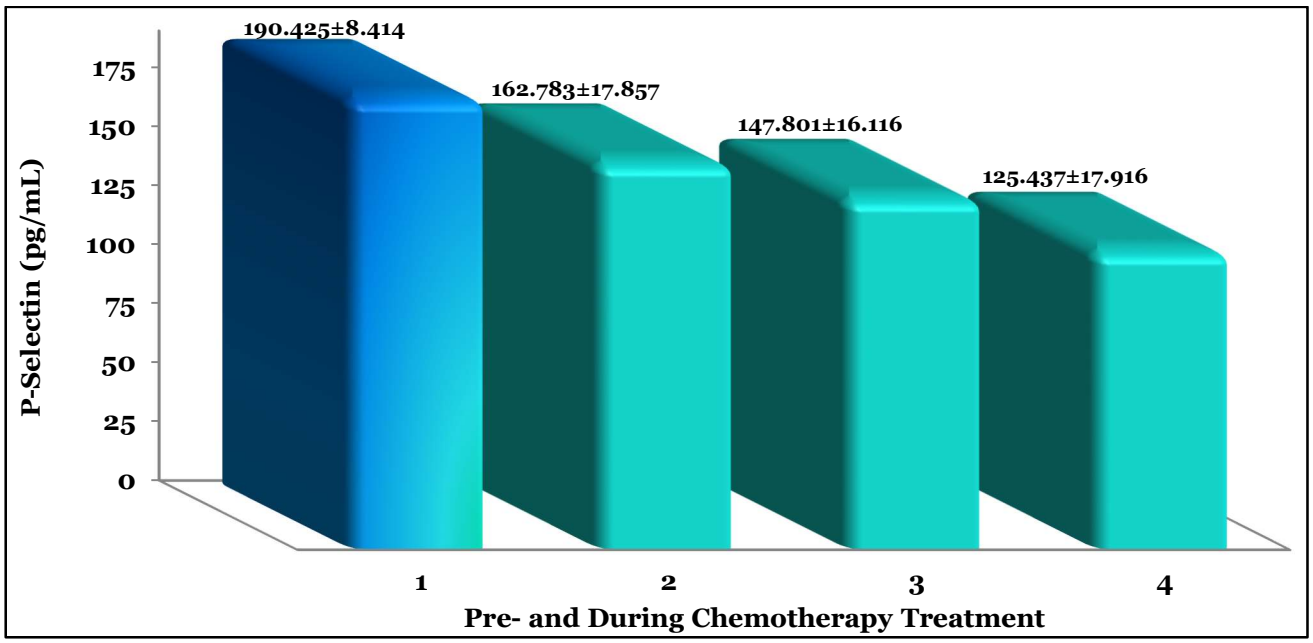

Figure 6: Comparison Levels of P-Selectin in Cancerous Patients at Pre and Post Chemotherapy Treatment

Selectins play an important role in the stage of vascular and cancer cell entrapment in the targeted metastatic tissues through the formation of metastases[31]. Significantly higher levels of P-Selectin concentration were observed in the serum of women with endometrial cancer (EC) compared to the control group in a study by Dominika, *et al.*, this study also confirmed the occurrence of high levels of P-Selectin concentration in the serum of patients with various malignancies including breast cancer[32]. Selectins are present on the surface of lymphocytes, activated endothelial cells and activated platelets and play an important role in cancer through the inflammatory response. Studies have also shown that selectin ligands are present in some cancer cells. These ligands are present on some breast cancer cells and can roll and bind to the surface of platelets under physiological conditions in the form of glycoproteins. There were also statistical differences in a study conducted during the interaction of P-selectin with breast cancer cells expressing Mac-2BP[33]. The finding of the present study agrees with the results of previous studies, so it indicates that P-Selectin can be appointed as predicting, diagnostic and following marker for breast malignancy[34].

Study of the Relationship between C-Type Lectin Domain Family 8 Members and P-Selectin in Malignant Breast Tumors, Benign Breast Tumors, and Healthy Controls

Significant positive correlations were observed when studying the relationship between CLEC8A and P-Selectin in the group of patients with breast cancer ($r = 0.712$ at $p<0.005$) and the group of healthy females ($r = 0.646$ at $p<0.005$), as shown in **Figures 7A** and **7C**, respectively.

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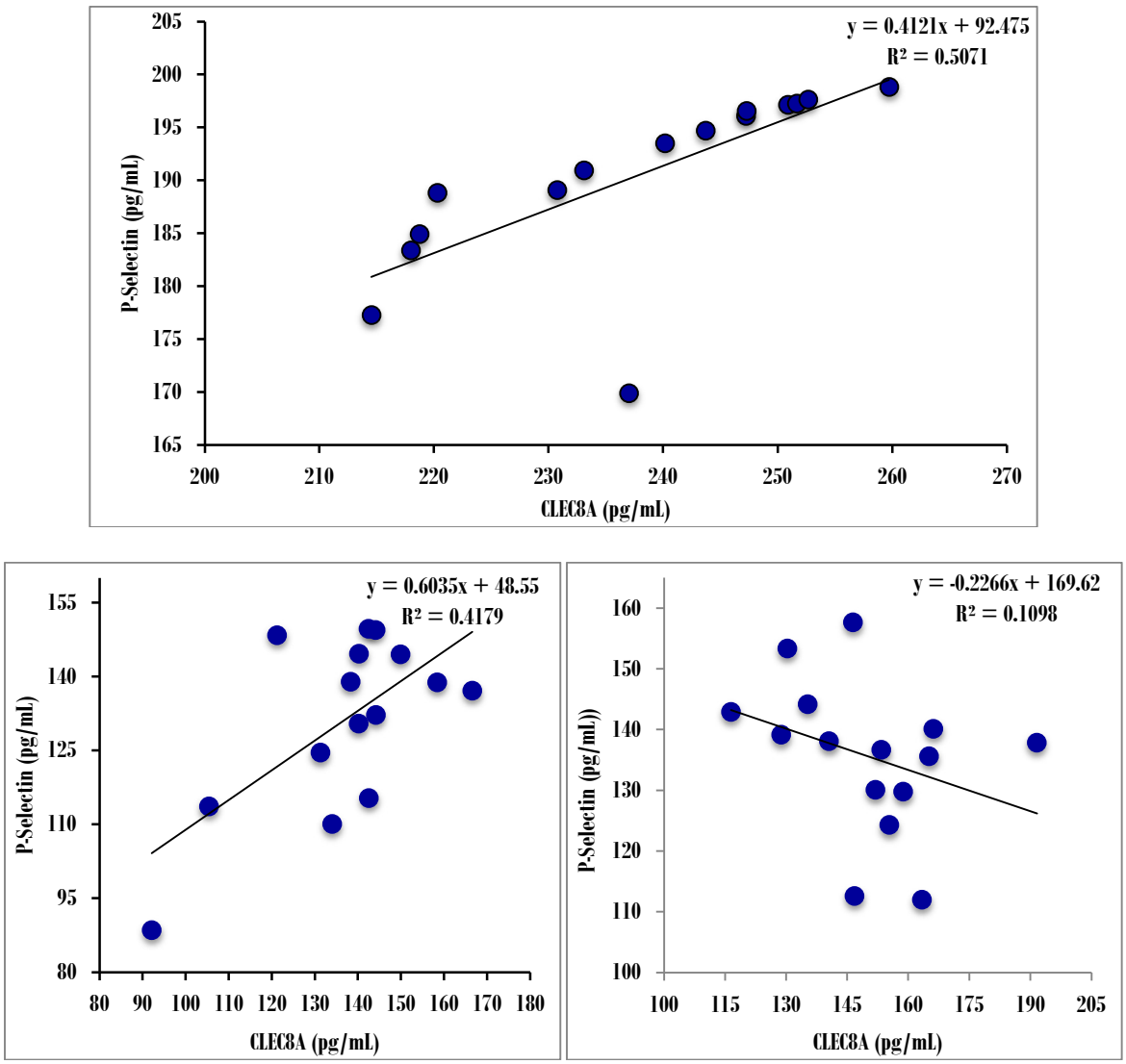

Figure 7: Correlation of CLEC8A to P-Selectin in(A) Cancer Tumor, (B) Benign Tumor, and (C) Healthy Control

In contrast to the previous results, a statistically insignificant negative correlation was observed in the group of patients with benign breast tumor when evaluating the relationship between CLEC8A and P-Selectin, as shown in **Figure 7B**.

Sensitivity and Specificity of the Evaluated Parameters

Sensitivity is known as the true positive rate or the probability of detection, it measures the proportion of positives that are correctly identified. Specificity is known as the true negative rate; it measures the proportion of negatives that are correctly identified.The calculation of sensitivity and specificity is used for assessing the efficiency of the tested parameters to suggest them as diagnostic markers.The diagnostic efficiency of the included criteria in this work were evaluated by applying the receiver operating characteristic (ROC) as demonstrated in Figurers 8, and 9 for CLEC8A, and P-Selectin; respectively.

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Table 5 shows the area under the curve and the cut-off value for the criteria evaluated in the current study. The individual efficiency (sensitivity) of the criteria evaluated in the current study for distinguishing between cancerous and benign breast tumors reached the highest level (100%), while the study established the highest specificity (100%) for both CLEC8A and P-Selectin.

Table 5: Receiver Operating Characteristic Analysis of CLEC8A and P-Selectin as Prognostic Tumor Markers for Breast Cancer

Criteria	AUC	SE	p-value	Cutoff value	Sensitivity%	Specificity%	CI (95%)
CLEC8A	1.000	0.000	0.000	203.069	100	100	1.000-1.000
P-Selectin	1.000	0.000	0.000	163.784	100	100	1.000-1.000

AUC: Area Under Curve, SE: Standard Error

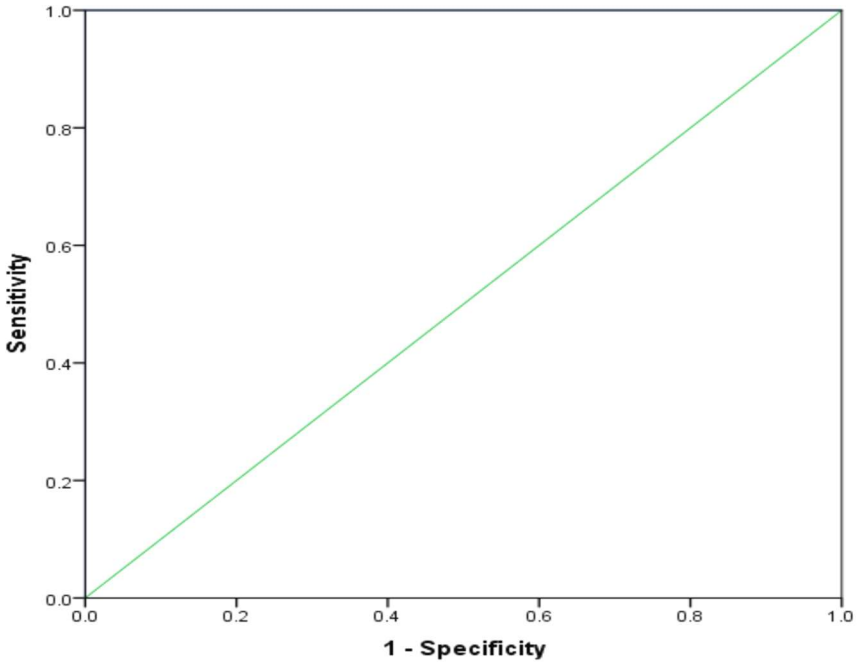

Figure 8: Receiver Operating Characteristic Curve forCLEC8A

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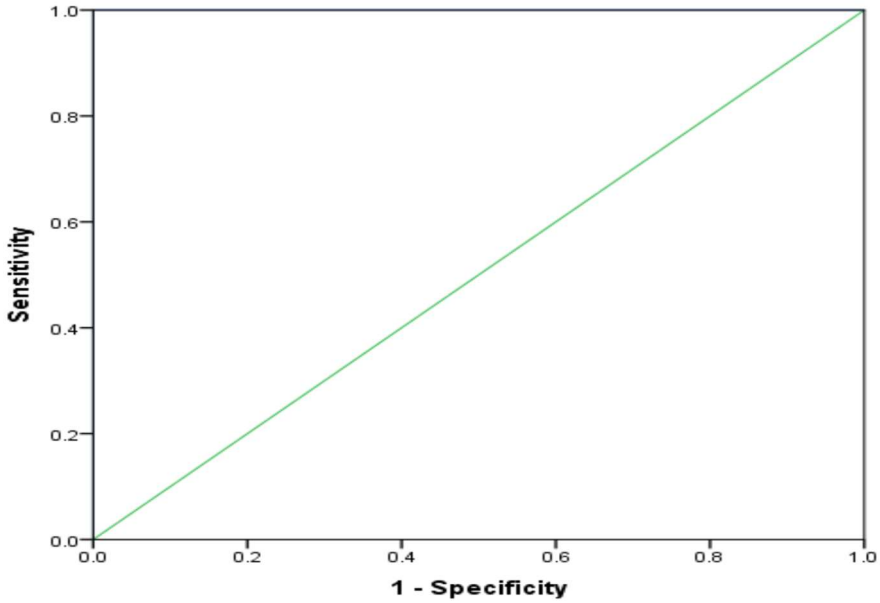

Figure 9: Receiver Operating Characteristic Curve ofP-Selectin

The combined sensitivity of the four criteria CLEC8A and P-Selectin were examined in the breast tumor, as shown in **Table 6**. All criteria in this study showed a similar maximum sensitivity for each parameters (100%).

Table 6: Combined Sensitivity of the Evaluated Parameters

Criteria	CLEC8A	P-Selectin
CLEC8A	100	100
P-Selectin		100

The absolute sensitivity of the criteria evaluated in the current work provides them as tools to differentiate between malignant and benign breast tumors. Moreover, the results of the work indicate the possibility of employing these criteria in evaluating the response of cancer patients to chemotherapy. Therefore, the current study provides four excellent tools for diagnosing breast cancer and following up the recovery phase, which helps the specialist physician in designing the treatment strategy provided and evaluating the level of recovery.

When calculating the value of the combined specificity, it was found that it reached 100% when calculated with both CLEC8A and P-Selectin, while the study indicated that the percentage of specificity reached 93% when evaluating both lectins together, as shown in **Table 7**.

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Table 7: Combined Specificity of the Evaluated Parameters

Criteria	CLEC8A	P-Selectin
CLEC8A	100	100
P-Selectin		100

Accordingly, the results of the present work indicate the possibility of using one of the two criteria, CLEC8A or P-Selectin, alone to infer a benign breast tumor, as the levels of these two criteria remain within their limits in the sera of healthy people, and thus it can be asserted that the detected tumor is a non-cancerous tumor.

Referensers

(1) Maleki EH, Bahrami AR, Matin MM. Cancer cell cycle heterogeneity as a critical determinant of therapeutic resistance. Genes Dis. 2024;11(1):189–204.
(2) Obeagu EI, Obeagu GU. Breast cancer: A review of risk factors and diagnosis. Medicine. 2024;103(3): e36905.
(3) Valavanidis A. Cancer Cases and Deaths Worldwide Attributable to Potentially Modifiable Risk Factors.
(4) Islami F, Marlow EC, Thomson B, McCullough ML, Rumgay H, Gapstur SM, *et al.,* Proportion and number of cancer cases and deaths attributable to potentially modifiable risk factors in the United States, 2019. CA Cancer J Clin. 2024;74(5):405–32.
(5) Bakhtar O. Pathology of Breast Cancer: Subtypes, Receptors, Biologic Markers, and Staging. Surgical Clinics. 2023;103(1):1–15.
(6) Wimann SC, Williams CE, Guth A, Whittington J. Breast and Oncology. In: The ABSITE Blueprints. Springer; 2023. p. 289–326.
(7) Fakhri N, Chad MA, Lahkim M, Houari A, Dehbi H, Belmouden A, *et al.,* Risk factors for breast cancer in women: an update review. Medical Oncology. 2022;39(12):197.
(8) Obeagu EI, Obeagu GU. Breast cancer: A review of risk factors and diagnosis. Medicine. 2024;103(3): e36905.
(9) Ding R, Liu Q, Yu J, Wang Y, Gao H, Kan H, *et al.,* Identification of Breast Cancer Subtypes by Integrating Genomic Analysis with the Immune Microenvironment. ACS Omega [Internet]. 2023 Apr 4;8(13):12217–31.
(10) Łukasiewicz S, Czeczelewski M, Forma A, Baj J, Sitarz R, Stanisławek A. Breast cancer—epidemiology, risk factors, classification, prognostic markers, and current treatment strategies—An updated review. Vol. 13, Cancers. MDPI; 2021.
(11) Naga Jyothi C, Rajesh B, Devi R, Kodandapani S, Ambroise M, Kiran CM, *et al.,* Amplification of C-MYC-shedding light on molecular landscape in human breast carcinoma. Naga Jyothi /AfrJBioSc. 2024;6(6).
(12) Akhmedov A, Sawamura T, Chen CH, Kraler S, Vdovenko D, Lüscher TF. Lectin-like oxidized low-density lipoprotein receptor-1 (LOX-1): a crucial driver of atherosclerotic cardiovascular disease. Eur Heart J. 2021;42(18):1797–807.
(13) Drouin M, Saenz J, Chiffoleau E. C-type lectin-like receptors: head or tail in cell death immunity. Front Immunol. 2020; 11:251.
(14) Kattoor AJ, Goel A, Mehta JL. LOX-1: regulation, signaling and its role in atherosclerosis. Antioxidants. 2019;8(7):218.

ISSN:1624-1940
http://magellanes.com/

(15) Akhmedov A, Sawamura T, Chen CH, Kraler S, Vdovenko D, Lüscher TF. Lectin-like oxidized low-density lipoprotein receptor-1 (LOX-1): a crucial driver of atherosclerotic cardiovascular disease. Eur Heart J. 2021;42(18):1797–807.
(16) Janiszewska M, Primi MC, Izard T. Cell adhesion in cancer: Beyond the migration of single cells. Journal of biological chemistry. 2020;295(8):2495–505.
(17) Yeini E, Satchi-Fainaro R. The role of P-selectin in cancer-associated thrombosis and beyond. Thromb Res. 2022;213: S22–8.
(18) Ferreira G, Taylor A, Mensah SA. Deciphering the triad of endothelial glycocalyx, von Willebrand Factor, and P-selectin in inflammation-induced coagulation. Front Cell Dev Biol. 2024; 12:1372355.
(19) Suzuki A, Tomita H, Okada H. Form follows function: the endothelial glycocalyx. Translational Research. 2022; 247:158–67.
(20) Moore KH, Murphy HA, George EM. The glycocalyx: a central regulator of vascular function. American Journal of Physiology-Regulatory, Integrative and Comparative Physiology. 2021;320(4): R508–18.
(21) Tvaroška I, Selvaraj C, Koča J. Selectins—the two Dr. Jekyll and Mr. Hyde faces of adhesion molecules—a review. Molecules. 2020;25(12):2835.
(22) Katalinic A, Eisemann N, Kraywinkel K, Noftz MR, Hübner J. Breast cancer incidence and mortality before and after implementation of the German mammography screening program. Int J Cancer. 2020 Aug 1;147(3):709–18.
(23) Dickson-Swift V, Adams J, Spelten E, Blackberry I, Wilson C, Yuen E. Breast cancer screening motivation and behaviours of women aged over 75 years: a scoping review. BMC Womens Health. 2024 Dec 1;24(1).
(24) Svanøe AA, Humlevik ROC, Knutsvik G, Sæle AKM, Askeland C, Ingebriktsen LM, *et al.,* Age-related phenotypes in breast cancer: A population-based study. Int J Cancer. 2024 Jun 1;154(11):2014–24.
(25) Tzenios N, Tazanios ME, Chahine M. The impact of BMI on breast cancer - an updated systematic review and meta-analysis. Medicine (United States). 2024 Feb 2;103(5): E36831.
(26) Blair CK, Wiggins CL, Nibbe AM, Storlie CB, Prossnitz ER, Royce M, *et al.,* Obesity and survival among a cohort of breast cancer patients is partially mediated by tumor characteristics. NPJ Breast Cancer. 2019 Dec 1;5(1).
(27) Harborg S, Zachariae R, Olsen J, Johannsen M, Cronin-Fenton D, Bøggild H, *et al.,* Overweight and prognosis in triple-negative breast cancer patients: a systematic review and meta-analysis. Vol. 7, npj Breast Cancer. Nature Research; 2021.
(28) Truthe S, Klassert TE, Schmelz S, Jonigk D, Blankenfeldt W, Slevogt H. Role of Lectin-Like Oxidized Low-Density Lipoprotein Receptor-1 in Inflammation and Pathogen-Associated Interactions. Vol. 16, Journal of Innate Immunity. S. Karger AG; 2024. p. 105–32.
(29) Moghadam SG, Pour ME, Alavizadeh SH, Kesharwani P, Sahebkar A. The association between oxidized low-density lipoprotein and cancer: An emerging targeted therapeutic approach? Bioorg Med Chem Lett. 2024;129762.
(30) Deng CF, Zhu N, Zhao TJ, Li HF, Gu J, Liao DF, *et al.,* Involvement of LDL and ox-LDL in cancer development and its therapeutical potential. Front Oncol. 2022; 12:803473.
(31) Shi Q, Ji T, Tang X, Guo W. The role of tumor-platelet interplay and micro tumor thrombi during hematogenous tumor metastasis. Cellular Oncology. 2023;46(3):521–32.
(32) Wang TF, Liou YS, Yang SH, Lin GL, Chiang YW, Lien TS, *et al.,* Platelet-derived circulating soluble P-selectin is sufficient to induce hematopoietic stem cell mobilization. Stem Cell Res Ther. 2023;14(1):300.

ISSN:1624-1940
http://magellanes.com/

(33) Kim B, Park B, You S, Jung SH, Lee S, Lim K, *et al.,* Cell membrane camouflaged nanoparticle strategy and its application in brain disease: a review. J Pharm Investig. 2024;1-17.
(34) Wang, Wei, *et al.,* High platelet-lymphocyte ratio may predict poor therapeutic response in breast cancer patients treated with neoadjuvant chemotherapy. 2024.